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A CONTRIBUTION TO THE CLINICAL KNOWLEDGE OF TEXAS FEVER.*

C. C. GUTHRIE,
University of Chicago.

TEXAS fever, as is well known, is a malarial type of disease which attacks cattle in southern latitudes. It is characterized, as its name implies, by an elevation of body temperature. Hemoglobinuria commonly occurs.

The direct cause is a microparasite named (1889) from its appearance *Pyrosoma bigeminum* by Dr. Theobald Smith,[1] its discoverer.

It was shown in 1889–1890 by Kilborne[2] that a cattle tick of a particular variety (*Boophilis bovis*) is the natural transmitting agent.

These observers also showed that the disease may be produced at will, in a susceptible animal, by subcutaneously introducing blood from an "immune" animal, that is one that has apparently recovered from an attack of the disease.

Attempts to produce an antitoxic serum have proven unsuccessful.[3]

It has been observed that the disease produced in northern cattle by inoculation (injection of virulent blood) is less fatal than when it is acquired in the natural way, and that animals thus treated have a high degree of "immunity" after recovery.[4] Work along this line has largely been developed by Dr. J. W. Connaway of the Missouri Experiment Station and economic inoculation has been practiced by him with great success, the details of which may be found in the publications referred to. Dr. Conna-

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[1] SMITH AND KILBORNE, *U. S. Bureau of Animal Industry*, *Bull.* 1893, 1, p. 177.

[2] *Loc. cit.*

[3] *Mo. Exp. Sta. Bull.*, 1897, 37; *Miss. Exp. Sta. Bull.*, 1898, 42; *ibid.*, 1899, 48.

[4] SMITH AND KILBORNE, *loc. cit.*; J. W. CONNAWAY, *Mo. Exp. Sta. Bull.* 37, 1897; CONNAWAY AND FRANCIS, *Mo. Exp. Sta. Bull.*, 1899, 48, and *Texas Exp. Sta. Bull.*, 1899, 53; M. FRANCIS, *Texas Exp. Sta. Bull.* 1902, 63; J. S. HUNT, *Report of the Inspector of Queensland Stock Institute*, 1897-8.

way published graphic records of the fever of seven bulls[1] and Dr. Francis a chart showing the course of the temperature and hematokrit readings of five heifers and one bull.[2]

Since these and other exact clinical observations hitherto made were not considered sufficiently exhaustive, I made a detailed study of the curve of temperature and the relative volume of corpuscles and plasma, as determined by the hematokrit, in 62 animals of different sex and various ages with a view of determining (*a*) the average curve of temperature and hematokrit readings, and (*b*) the influence of sex and age on these curves and the course of the fever in general. In addition to the temperature and hematokrit readings, in another animal, largely with the view of elucidating the remarkable hemolysis and hemoglobinuria already referred to, I investigated the chemical and morphological changes in the blood and physico-chemical changes in the blood and urine (Cow 2). I also made attempts to determine how long the blood retains its virulence when kept aseptically outside of the body at room temperature.

Chart 1 showing the average course of the temperature and hematokrit readings produced in cattle by inoculation is based on data gathered in eastern Kansas during July and August 1901 from a herd consisting of 62 head of registered Herefords. Of the cattle 41 were bulls and 21 heifers. They may be divided into four groups:

I. Thirty-five bulls ranging in age from 12 to 16 months;
II. Six bull calves, from about 6 weeks to 4 months;
III. Sixteen heifers, from about 12 to 16 months;
IV. Five heifer calves, from about 3 to 5 months.

On June 27 each animal of the herd received subcutaneously from 0.75 to 1.0 c.cm. of defibrinated blood obtained from an immune yearling steer. The mother of the steer was a small black Texas cow which was shipped to the Missouri Experiment Station August 1896. Before being shipped she had been dipped to free her from ticks and subsequently was several times inoculated from animals having the fever. Kept afterwards for two summers in a tick infested pasture, she gave evidence of no reaction. The sire of the steer was an immune registered Jersey. When a few weeks old, the steer was inoculated with blood from his mother and marked symptoms of fever appeared. Afterward he received blood from other animals immune to Texas fever. Ultimately he ceased to react to inoculation.

[1] *Mo. Exp. Sta. Bull.*, 1899, 48, p. 26.

[2] *Loc. cit.*, p. 65.

The inoculated cattle were kept in stalls and fed a ration consisting of a mixture of ground oats, corn and wheat, with the exception of Groups II and IV, which in addition nursed. For roughness they were given new alfalfa hay. At night, owing to the heat and their need for water and exercise, they were turned into small lots adjoining the barn. During the day they were frequently sprayed with a preparation that kept from them the troublesome "horn flies."

The sick animals were sprayed with water to reduce their temperature and were given saline purgatives together with copious rectal injections of water to keep the bowels open. Temperature records were taken between six and eight o'clock in the morning and hematokrit readings from two to five in the afternoon.

In Chart 1 are displayed the curves of temperature and hematokrit readings determined by averaging results (1) of all the animals, (2) of the heifers, (3) of the bulls.

The inoculation fever may be divided into five periods, viz:

(1) *Primary incubation period*, from inoculation to the onset of fever (first to ninth day) is nine days long. During this time the percentage of sediment in the blood slightly decreases.

(2) *Primary fever period*, from the onset of the fever until the beginning of the intermediate period (9th to 18th day) is nine days long. The maximum temperature is reached on the sixth day, thus giving a relatively long *initial stage*. The *fastigium* is very short, being less than one day in length. The *stage of defervescence* is three days in length. Thus the fever terminates by *lysis*.

During the initial stage, the blood sediment sharply decreases. This is succeeded by a gradual increase during the latter part of the stage of defervescence.

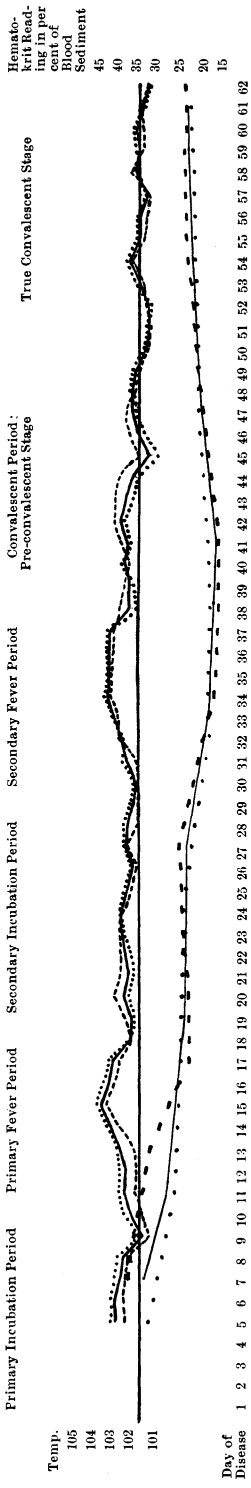

CHART 1.—The light lines mainly above the abscissa, represent the temperatures; the heavy lines mainly below the abscissa, the hematokrit readings. (1) The continuous lines represent the average of all the animals; (2) the dotted lines, the averages of the bulls; (3) the interrupted lines, the averages of the heifers.

(3) The term *secondary incubation period* may be appropriately applied to the interval from the end of the primary until the beginning of the secondary fever period (18th to 30th day). This is 12 days long. During this time the temperature is normal or slightly hypernormal. The blood sediment slowly increases during this period.

(4) *The secondary fever period*, from the end of the intermediate to the beginning of the convalescent period (30th to 38th day) is eight days long.

The *initial stage* is four days long. The maximum temperature for the period occurs on the last day of this stage and is slightly less than for the primary fever period. The *fastigium* is of three days duration. During this stage the morning temperatures remain practically constant. The stage of *defervescence* is only one day long, thus the fever terminates by *crisis*. During the initial stage, the blood sediment decreases more sharply and to a greater degree than in the corresponding stage of the primary fever. This decrease continues during the fastigium but during defervescence it begins again gradually to increase.

(5) *The convalescent period* may be divided into two stages:

(*a*) What may be called the *preconvalescent stage*, which lasts from the end of the secondary fever to the beginning of true convalescence (38th to about the 51st day) is about 13 days long. It is characterized by irregular hyper- and subnormal temperatures. The blood sediment continues to gradually increase.

(*b*) *The true convalescent stage*, which begins with the termination of the preconvalescent stage and terminates with the complete recovery of the animal. It may be roughly stated as being from 30 to 90 days in length. During this stage the temperature is normal and the blood sediment gradually returns to normal.

Charts of the individuals comprising the herd show that the course of the disease is often atypical as judged by the temperature and blood sediment records. In the case of the former the widest variations occurred, the curves showing in some cases practically no rise in temperature, while in others, the elevation was continuous throughout the time corresponding to the primary fever, secondary incubation and secondary fever periods. In some the primary elevation was absent; in others the secondary. When both elevations occurred, either might be the higher.

The curve of blood sediment was very constant as regards the primary and secondary decreases, both being present in every case. Variations as to time and amount of decrease are not uncommon.

The influence of age and sex on *the course* of (*a*) *temperature* and (*b*) *blood sediment* is shown in Table 1.

TABLE 1.

GROUPS GIVING SEX AND AGES	MAXIMUM PRIMARY FEVER	MAXIMUM SECONDA'Y FEVER	PER CENT OF BLOOD SEDIMENT			
			Max. for Prim. Inc. Period	Min. for Prim. Fev. Period	Max. for Sec. Inc. Period	Min. for Sec. Fever Period
I. Bulls: Thirty-five young adults.....	104.9	104.8	31.0	23.4	25.2	12.4
II. Bulls: Six calves	104.2	105.0	35.0	28.1	29.6	12.3
III. Heifers: Sixteen young adults.....	104.8	104.7	37.3	23.0	27.5	14.0
IV. Heifers: Five calves............	104.7	104.4	39.0	26.0	27.3	13.7

(*a*) Only in the group of bull calves does any considerable difference occur between the average maximum temperature of the primary and secondary fever periods. It appears that the primary fever was less in the calves than in the older bulls and heifers, and that of all the groups, the heifer calves had somewhat the lowest fever during both periods.

(*b*) During the *primary incubation period* the average maximum percentage of blood sediment was higher in both groups of heifers than in either of the groups of bulls. Also in both sexes it was higher in the calves than in the older animals.

In the *primary fever period* the lowest average minimum occurs in the older animals, being slightly less in the group of older heifers than in the older bulls. Also, in the calves, the average in the heifers was slightly lower than in the bulls. The percentage of decrease from the prior reading was also greater in both groups of heifers than in the bull groups. In both sexes, the decrease was greater in the older animals than in the calves.

During the *secondary incubation period* the bull calves showed the largest amount of blood sediment and the older bulls the smallest of any group. In the heifers it was slightly higher in the older animals than in the calves, and both groups were intermediate between the two bull groups. The percentage of increase was in both sexes greater, as might be expected from the previous decrease, in the older animals than in the calves, being much greater, however, in the heifers. In the calves, the increase was slightly greater in the bulls.

During the *secondary fever period* the minimum amount of blood sediment was lower and percentage of decrease from the previous readings greater in both groups of bulls than in either of the groups of heifers. In both sex groups, the minimium sediment in calves was slightly lower than in the older animals.

Influence of age and sex on nutrition and recovery is well marked. It is known that young animals stand the disease much better than old ones and that mortality is less among nursing calves than any other class.[1]

The calves, although showing high fever and low hematokrit readings, withstood the fever far better than the older animals. The bull calves rarely refused to nurse. The heifer calves also ate much more regularly than did the older animals. The loss of flesh was much less in the calves than in the older cattle.

That the disease was better withstood by the heifers in general is indicated by the following facts:

1) The mortality among the 41 bulls was 7.3 per cent; while none of the 21 heifers died.

From this it must not be inferred that the inoculation fever is never fatal to heifers. The mortality, however, is considerably lower than among bulls.

2) The disease did not have so great a tendency, in the heifers as in the bulls, to become chronic, i.e. irregular and protracted to beyond the usual time of termination.

3) Inactivity of the bowels and suppression of the urine, conditions often seen in the severer type of fever, was less frequent among the heifers than among the bulls.

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[1] CONNAWAY, *Mo. Exp. Sta. Bull*, 1899, 48, p. 60.

4) That the nutrition of the heifers was less seriously impaired than that of the bulls, was shown by the former being less emaciated, their recovery more rapid and by their eating more regularly during the disease.

The period at which death is most liable to occur, according to these observations, is during the secondary fever period, from the 36th to 40th days. But, as Dr. Connaway[1] has stated it may occur during the primary fever period. There was also one death in my series during the convalescent period. The following protocols give data bearing on this point.

Three of the oldest bulls died of the disease viz., No. 6 on the 16th, No. 71 on the 36th and No. 69 on the 37th day of inoculation, Bull No. 6 died at a time corresponding to the stage of defervescence of the primary fever. The fever began on the seventh and rose irregularly to 106.4 on the 13th day. The fastigium lasted two days, or until the 15th during which time the maximum temperature was 106.6. The fever terminated by crisis, a sharp fall in the temperature from 106.2 to 102.3 occurring the day prior to death. On the fifth day of inoculation, the hematokrit showed 29.0 per cent of sediment; on the 11th, which was the fifth day of the initial stage, 25.0 per cent. From this time onward until the 15th day, when the reading was 10.0 per cent, the decrease was sharp and fairly constant from day to day. The 16th, the day death occurred, the reading remained at 10.0 per cent. This would indicate that death was due to some cause other than diminution of the blood sediment, particularly as in several other similar animals, inoculated at the same time, recovery occurred after the hematokrit had shown only seven per cent of sediment.

Bull 71 died on the second day of the stage of defervescence of the secondary fever period. The temperature curve is unusual in this case. The temperature during the incubation period was variable. The primary fever period began on the 13th and terminated on the 18th day. The inital stage was two days in length, during which time the temperature rose gradually from 101.6 to 103.6. During the secondary incubation period, the temperature was normal, ranging from 102.0 to 102.6 until the last day, when it sank to 101.0. The secondary fever period began on the 30th day. The initial stage terminated on the 35th during which time the temperature irregularly rose to 104.8. From this point it gradually fell for two days until it was 101.5 which was the day of the death of the animal.

The blood sediment showed no decrease up to the 15th, the day of maximum primary fever, when it was 30.0 per cent. On the 22d, it showed a decrease to 26.0 per cent. On the 29th, which was the day preceding the secondary fever, it was 25.0 per cent. The next and last reading, which was on the 36th, was 10.0 per cent.

This shows that the primary loss of sediment was very slight and even more greatly delayed than the primary elevation of temperature.

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[1] *Mo. Exp. Sta. Bull.*, 1899, 48, p. 58.

Bull 69 exhibited temperature and blood sediment curves very like those of No. 71; therefore a detailed description is unnecessary. The only essential difference occurs in the temperature curve of the secondary fever, which began on the 28th day. The temperature rose fairly regularly to 104.5 on the 32d. It remained practically constant until the 35th, when it was 104.0, giving a fastigium three days long. The fever terminated by crisis, falling to 100.6 on the next, the 36th and last day. The blood sediment was 26.5 per cent on the 29th and 10.0 per cent on the 36th.

In addition to these three animals, Cow No. 2 (temperature and hematokrit records are given in Table 3 and Chart 2), died from inoculation *, death occurring, after well marked primary and secondary periods, on the 40th day, corresponding to the fifth day of the preconvalescent stage.

Of 63 head of cattle of different sex and ages, four deaths occurred from inoculation as follows: one at the end of the primary fever period; two at the end of the secondary fever period and one during the beginning of the preconvalescent stage. These figures would indicate that the period of greatest mortality is between the 36th and 40th day after inoculation.

Through the courtesy of the Missouri Experiment Station, the cows used in the following experiments were placed at my disposal during the summer of 1903.

Cow 1, the immune animal used for supplying the blood for inoculation, was the Texan described on p. 530. She was in good condition, being intermediate between the other two.

Cow 2, used for inoculation, was a well bred Jersey about twelve years old. Her condition was medium and she was in the third month of pregnancy.

Cow 3, used as a control, was a registered Jersey belonging to the dairy herd. She was eight years old, in excellent condition and in the third month of pregnancy.

From the data obtained, Table 2, showing the *individual* and *average* normals, was prepared.

All the cows were kept in a good blue-grass pasture which had an abundance of shade and running water. Throughout the period of the experiment, they had access to salt and hay.

Blood for microscopical examination was obtained from the three animals on the afternoon of July 13, by puncturing the marginal ear vein. On the afternoon of the 14th about 1000 c.cm. of blood was aseptically drawn from each by inserting a trochar into the external jugular vein and about half of this

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* In considering the mortality resulting from inoculation in these experiments from an economic standpoint it should be borne in mind that aged cows like Cow 2 almost invariably die.

TABLE 2.

Number of Cow	Hematokrit per cent of Sediment	Number of Red Corpuscles per cu. mm.	Volume Index of Red Corpuscles	Number White Corpuscles per cu. mm.	Proportion of White to Red Corpuscles	Hemoglobin Estimation (Tallquist)	Hemoglobin Estimation (Dare)
1	37.0	6,124,000	0.596	5,520	1:1109	95.0	50.0
2	36.0	5,591.000	0.648	4,760	1:1174	97.5	64.5
3	45.0	6,205,000	0.725	3,400	1:1823	100.0	60.0
Average	39.3	5,773,000	0.656	4,560	1:1368	97.5	58.1

Number of Cow	Hemoglobin Estimation (Sp. Gr. Method)	Color Index (Hb. Estimation by Tallquist)	Color Index (Hb. Estimation by Dare)	Blood Sedime't Hemoglobin Ratio	Sp. Gr. of Blood	Sp. Gr. of Serum	Sp. Gr. of Blood Sedime't	Osmotic Resistance of the Red Corpuscles
1	85.0	0.778	0.409	1.28	1.0570	1.0330	1.096	0.54
2	74.0	0.878	0.581	1.35	1.0545	1.0350	1.088	0.54
3	95.0	0.806	0.483	1.11	1.0600	1.0300	1.096	0.54
Average	84.6	0.820	0.491	1.24	1.0571	1.0326	1.093	0.54

Number of Cow	Coagulation time of Blood in Seconds	Fibrin of Blood	Alkalinity of Blood	Alkalinity of Serum	Chlorides of Serum	Diastatic Ferment of Blood	Diastatic Ferment of Serum	Coagulable Proteid of Serum
1	190	0.704	133.2	77.3	0.500	0.024	0.025	....
2	168	0.692	98.6	95.9	0.504	0.017	0.018	4.95
3	168	0.350	103.9	79.9	0.546	0.023	0.021	....
Average	175.3	0.581	111.9	84.4	0.516	0.021	0.022	....

Number of Cow	Δ of Blood	Δ of Serum	Relative Electrical Conductivity of Blood	Relative Electrical Conductivity of Serum	Sp. Gr. Urine	Alkalinity of Urine	Chlorides of Urine	Urea of Urine	Δ of Urine
1	0.549	0.549	127.2	112.5	....	....	....	.....	.....
2	0.578	0.569	100.0	100.0	1.031	94.0	0.55	0.019	1.947
3	0.581	0.555	136.3	112.5	1.022	55.0	0.76	0.011	.....
Average	0.569	0.558	.....	.....	.....	....	....	.....	.....

was, in each case, defribrinated and the rest allowed to clot. The various portions of blood were sealed in sterile glass jars which were kept on ice until the examinations and analyses were finished

The hematokrit readings were made independently by two individuals, the average of the two readings being recorded. In no instance did the difference in the readings amount to more than 0.25 as read on the scale of the tube. Daland's hematokrit was employed which gave a speed of 10,000 revolutions per minute.

The number of red corpuscles was determined in the blood obtained before coagulation from the ear and in defibrinated blood drawn from the jugular vein. The differences, if any, are negligible. The Thoma-Zeiss apparatus with the Türck ruling was employed. Nine-tenths per cent NaCl was used for diluting the blood. It is considered superior to more concentrated solutions often recommended for this purpose, owing to the fact that I found the counting easier when the corpuscles were uncrenated. Possible error due to the more rapid settling of the corpuscles that occurred, than when stronger solutions are employed, was avoided as far as possible by mounting rapidly. The greatest care was exercised in determining the exact dilution and in making perfect mounts. A block of 36 small squares was counted in each corner of the ruled field and another as near the center as possible. This gave the total for 180 squares. Another mount was then made and the count repeated, the result of which was averaged with the first, and the total number per cu. mm. calculated. The variation between the two counts was always very small. In a few instances the counts were identical.

The volume index of the red corpuscles was determined by the Capps' method,[1] the formula used being the per cent normal hematokrit reading divided by the per cent normal number, and expressing the result decimally.*

The number of white corpuscles was determined by means of the same instrument used in the red count, only the appropriate pipette was used. Three per cent acetic acid was used for laking the red corpuscles and diluting. The same care was exercised as in the case of the red count and the same method used excepting 2304 small squares were counted each of the two counts, giving a total of 4608.

The percentage of hemoglobin was determined by two methods: Tallquist's and Dare's. Readings were taken by three individuals, the recorded number being always an average of the three readings. In general, the individual variations were much less with the Tallquist than with the Dare method; also the readings by the former method agree more closely with the amount of hemaglobin indicated by the hematokrit, red count, and specific gravity of the blood.[2] But neither method is sufficiently accurate to give really reliable information as regards absolute amount of hemoglobin.

The color index was calculated in the usual manner, i. e. by dividing the percentage normal number of red corpuscles into the percentage of normal hemoglobin and expressing the result decimally. The results are recorded in two columns in the table, one based on the hemoglobin estimation by Tallquist's and the other by Dare's method. The results are considered to be of little actual value, the main reasons for including them in the tables being for completeness and reference. They are not sufficiently consistent in Cow 2, (Table 3) to render it possible to draw any general conclusions from them, doubtless because the average size of the corpuscles was no longer normal. To meet this difficulty, another factor called *the blood sediment Hb ratio*, obtained by dividing the percentage normal hemoglobin (Tallquist's method) by the percentage normal total volume of sediment as determined by the hematokrit, is used. By reference to Table 3, it may be observed that this factor more nearly expresses the condition of the blood coloring matter, judging mainly from the hemoglobin estimations, specific gravities and hemotokrit readings, than do the color indices.

The specific gravity of the blood was determined by Hammerschlag's method the samples consisting of defibrinated blood from the jugular.

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* In calculating the volume and color indices, the normal hematokrit reading was taken as 50; the number of red corpuscles as 5,000,000 and the hemoglobin as 100.

[1] CAPPS, *Jour. Amer. Med. Assn.*, Feb. 16, 1901, 34, p. 464.

[2] Hammerschlag's method. See EWING, *Clin. Diag.*, 2nd ed., 1903, p. 57, 58.

The specific gravity of the serum, in addition to the above method was determined by a hydrometer, the results of the two methods being practically identical.

The specific gravity of the blood sediment, the term applied to the factor used to indicate the richness of the blood sediment in hemoglobin, was calculated by the following formula :

$$\text{Sp. Gr of Sediment} = \frac{(100 \times \text{Sp. Gr. of blood}) - (\text{percentage of serum} \times \text{Sp. Gr. of the serum})}{\text{percentage of sediment}}$$

Where absolute rather than comparative results are desired, of course the hematokrit readings of sediment and serum must be corrected for the serum which remains in the interstices of the former, even after prolonged centrifugalization. Ordinarily this would certainly not amount to more than 10 per cent of serum for Eykman found only about 10 per cent of serum in the blood sediment centrifugalized at a speed of 2,600 per minute for one and one-half hours.

Stewart[1] found in 100 c.cm. of blood sediment after a second prolonged centrifugalization at about the same speed only 9.31 c.cm. of serum. Hamburger[2] points out that the amount of serum left in the sediment is proportional inversely to the speed of the hematokrit; therefore the sediment obtained with a speed of 10,000 revolutions per minute of the hematokrit contains in my experiments probably less than this amount of serum. In abnormal conditions the amount may vary, particularly any condition tending to alter the specific gravity of the sediment relative to the specific gravity of the plasma or serum or the character of the sediment, would be calculated to cause a variation. Without great accuracy in the hematokrit readings the factor is of little value as of course a small variation in the percentage of sediment would give a relatively large variation in the result.

In determining the *osmotic resistance of the red corpuscles* the method of Hamburger[3] was used. The readings were made at the end of one hour at room temperature. The figures given represent the minimum strength NaCl solution in which the corpuscles retained their hemoglobin.

The coagulation time was determined by inserting a trochar into the jugular vein, collecting the first 5 to 10 c.cm. of blood in a test tube and observing the time of beginning coagulation.

The amount of fibrin was determined by drawing and measuring a quantity of blood and then collecting the fibrin by whipping and straining through gauze. The fibrin was then washed in water until it became white after which it was preserved in three per cent formaldehyde. About a month after the last was obtained, the several amounts were removed from the formaldehyde and thoroughly washed in water and then extracted with alcohol-ether, after which it was dried at 110° C. and then weighed. In the table the amoumts represent the weight of dry fibrin in gms. per 100 c.cm. of blood.

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[1] STEWART, *Jour. Physiol.*, 1899, 24, p. 371.

[2] *Osmotischer Druckund Ionenlekre*, 1902, 1, p. 515.

[3] *Ibid.*, p. 61.

The alkalinity of the blood and serum was determined essentially by Engel's modification of Loewy's method.[1] One c.cm. of defibrinated blood being mixed with 20 c.cm. of distilled water and the mixture titrated with $\frac{n}{75}$ tartaric acid, the point of *slight* acidification being determined by testing with neutral litmus paper. In the case of the serum the titration was also performed after adding to the diluted serum a few drops of neutral litmus solution. The results by the two methods were the same. The blood and serum used were taken from the jars filled from the jugular, the test being made an hour after drawing the blood, excepting on the day of inoculation, owing to an unavoidable delay, 36 hours elapsed before the titration could be made. Therefore, the alkalinity given as normal in Table 2 is probably too low. The figures represent mg ms. NaOH per 100 c.cm.

The chlorides of the serum and urine were estimated by Salkowski's modification of Volhard's method[2] and expressed as gms. NaCl per 100 c.cm.

The diastatic ferment of the blood and serum was estimated as follows: To 25 c.cm. of a boiled starch solution in a test tube, made by adding two grams sugar free starch to 200 c.cm. of distilled water and boiling and then making up to 250 c.cm., one c.cm. of defibrinated blood or serum was added being deposited near the surface of the solution to insure thorough mixing. The sterile cotton plug was then replaced and the tube left at 25° C. for 48 hours at the end of which time its reducing power was estimated by Fehling's solution. The figures in the table represent the amount of reducing sugar in gms. found in 25 c.cm. of the starch solution. The tubes were set up within two hours of the time the blood was drawn.

The coagulable proteids of the serum and urine were determined quantitatively by the method of Roberts and Stolnikow as modified by Brandberg, which in numerous experiments in the laboratory has been found to give surprisingly accurate results. The figures are in terms of percentage.

Freezing point determinations were made by Beckman's method.[3] The average of three readings was recorded. In no case was the individual variation more than 0.005°. Usually it was less than 0.003°.

Relative electrical conductivity measurements were made by Kohlrausch's method[4] and the results are stated in the tables as percentage of conductivities on the day of inoculation, the measurements of the blood and serum on that day and of the urine on the third day being stated as 100 per cent in each case.

The urine was collected in the afternoon, the amount secured ranging from a few hundred to several thousand c.cm. No attempt was made to collect it for 24 hours at a time as it was impracticable under the circumstances.

The specific gravity of the urine was determined by means of an urinometer.

Urea was estimated in the urine by the sodium hypobromite method, the amount being expressed in terms of gms. per c.cm.

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[1] *Berl. klin. Wchnschr.*, 1898, 35, p. 308.

[2] *Ztschr. f. physiol. Chem.* 1882, 5, p. 285.

[3] *Ztschr. phys. Chem.*, 1888, 2, p. 638.

[4] KOHLRAUSCH AND HOLBORN, *Das Leitvesmögen und Elektrolyte*, 1898.

TABLE 3.

Day of Inocula'n	Temp. 8 A. M.	Hematokrit per cent of sediment	Number of red corpuscl's per cu. mm.	Volume index of red corpuscles	Number of white corpuscles per cu. mm.	Proportion of white to red corpuscles	Hemoglobin estimation (Tallquist)	Hemoglobin estimation (Dare)	Hemoglobin estimation (Sp. Gr. method)	Color index (Hb. estim. by Tallquist)	Color index (Hb. estim. by Dare)	Blood sediment Hb. ratio	Specific gravity of blood	Specific gravity of serum	Specific gravity of blood sediment	Osmotic resistance of red corpuscles	Coagulation time of blood in seconds	Fibrin of blood
−1		36.0			5040		97.50	80.00				1.35						
0			5,591,000	0.648	4760	1:1173	97.50	64.50	70.0	0.878	0.58	1.35	1.0545	1.0350	1.089	0.54*	168	0.692
3	101.1	36.0	5,451,000	0.660	3400	1:1602	97.50	73.00		0.894	0.69	1.35						
6	100.4	32.0	4,940,000	0.647			96.25	50.00		0.974	0.50	1.50						
10	101.4	31.0	4,120,000	0.752	2772	1:1487	97.50	52.00		1.183	0.61	1.57						
13	100.3	25.5	4,120,000	0.618	2177	1:1872	76 25	54.20		0.925	0.64	1.49						
16	102.0	20.0	3,506,000	0.571	1526	1:2337	67.50	38.25		0.962	0.51	1.68						
17	103.0	21.5	5,009,000	0.430	1464	1:3407	70.00	46.00	53.0	0.700	0.70	1.62	1.0475	1.0335	1.098	0.52	315	0.359
23	101.0	24.5	3,959,000	0.619	3611	1:1099	72.50			0.916		1.47						
36	98.9	11.0	1,026,000	1.073			23.75	9.00		1.153	0.43	1.07						
37	99.0	9.5	1,212,000	0.780	2000	1:606	23.25	7.50	32.0	0.960	0.31	1.22	1.0380		1.066		350	0.802
40	99.2													1.0350		0.48		

Day of inoculation	Alkalinity of blood	Alkalinity of serum	Chlorides of serum	Diastatic ferment of blood	Diastatic ferment of serum	Coagulable proteid of serum	Δ of blood	Δ of serum	Relative electrical conductivity of blood	Relative electrical conductivity of serum	Specific gravity of urine	Alkalinity of urine	Chlorides of urine	Urea of urine	Coagulable proteid in urine	Δ of urine	Relative electrical conductivity of urine
0		95.9	0.504	0.017	0.018	4.95	0.569	0.578	100.0	100.0							
3	98.6										1.032	94.4	0.55	0.020	none	1.947	100.0
13											1.024	384.0	0.40	0.028	none		
17	255.8	197.2	0.614	0.007	0.008		0.574	0.577	118.1	100.0	1.023	472.0	0.40	0.020	none	1.462	48.4
19											1.016	328.0	0.20	0.018	none		
37	223.9	169.5	0.614	0.012	0.013		0.671	0.684	136.3	118.7							
38											1.010	400.0	0.05	0.018	0.165	0.866	16.2
40			0.710			1.98					1.027	164.0	0.07	0.013	0.330		

The first line (−1) represents the day before, the second the day of, and the third the day after inoculation.
* On the 8th day it was the same.

TABLE 3—*Continued.*

Day of inoculation..........	3	4	5	6	7	8	9	10	11	12	13	14	15
Temp. 8 a. m...	101.1	100.7	100.4	100.4	100.0	100.1	101.0	101.4	101.0	100.9	100.3	101.0	102.3
Hematokrit readings.....	36.0		35.5	32.0		32.0		31.0		26.0	25.5		25.0
Day of inoculation..........	16	17	18	19	20	21	22	23	24	25	26	27	28
Temp. 8 a. m...	102.0	103.0	102.5	103.0	102.3	101.3	101.0	101.0	101.3	100.7	100.7	101.4	103.0
Hematokrit readings.....	20.0	21.5	21.0		21.0	24.0		24.5				20.5	
Day of inoculation..........	29	30	31	32	33	34	35	36	37	38	39	40	
Temp. 8 a. m...	103.2	105.0	105.2	104.0	102.4	101.7	98.3	98.9	99.0	100.8	100.7	99.2	
Hematokrit readings.....	18.0	17.0	14.5	14.0	12.0	9.0	10.5	11.0	9.5	9.0	9.0		

The alkalinity of the urine was determined by titration with $\frac{n}{10}$ oxalic acid, using blue litmus solution for the indicator. The results are expressed in terms of mg ms. NaOH per 100 c.cm.

On Cow 2, after inoculation with 25 c.cm. of defibrinated blood immediately after it was drawn from the immune Cow 1, temperature readings (rectal) were taken daily at 8 A. M. Hematokrit tests were made at 4 P. M. The results are displayed in Chart 2 and Table 3.

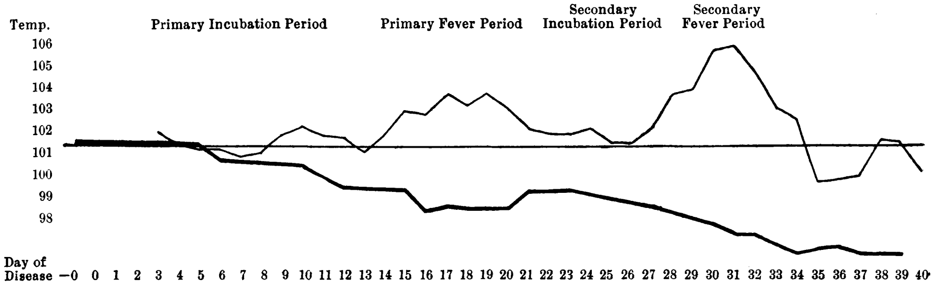

CHART 2.—The light continuous line represents the temperature; the heavy continuous line, the hematokrit readings.

The blood used for microscopical examination, etc., was obtained from the ear vein, excepting on the day of inoculation, and the 17th and 37th, when it was drawn from the jugular and defibrinated.

The serum was obtained from jugular blood on the day of inoculation, the 17th and 37th, and from blood from the heart at postmortem, which was within two hours of the death of the animal, on the 40th.

The urine was collected as described above excepting that in one instance a catheter was used and that on the 40th day it was taken from the bladder at the postmortem.

THE COURSE OF THE FEVER AS SHOWN BY THE CLINICAL FINDINGS.

THE TEMPERATURE READINGS.

The incubation period, 13 days in length, was normal for eight days. The first temperature was taken on the third day and it is slightly hypernormal, probably owing to the excitement of the animal which was unused to the manipulation. Subsequently readings on different mornings averaged about 100.5° and varied from each other by 0.0° to 0.4° until the eighth, when a period of hypernormal temperature began, which terminated on the 13th.

Between the 8th and 10th the temperature rose to 1.3° from which point it gradually decreased until the 13th, when the decrease amounted to 1.1°.

The primary fever was late, beginning on the 13th and lasting to the 22d, or nine days. The temperature increase from the 13th to the 15th was regular and amounted to 2.0°. On the 16th, a decrease of 0.5° was observed. On the 17th an increase of 1.0°, which was the maximum (103°) for the period.

The fastigium lasted from the 17th to the 19th. The fever during this time was of the remittent type. On the 18th, the temperature showed a decrease of 0.5°. The 19th showed a return to 103.0.

The stage of defervescence was from the 19th to the 22d. The first day the temperature decreased by 0.7°; the second, 1.0° and the third, 0.3°.

The secondary incubation period was four days in length, extending from the 22d to the 26th. Until the 24th, the temperature was practically constant. On the 25th,a drop of 0.4° occurred. The last day no change was observed.

The secondary fever period was about eight and one-half days in length, extending from the 26th to between the 34th and 35th. The *initial stage* was five days long, the increase in temperature being fairly regular until the maximum (105.2) was reached, which was 2.2° higher than in the primary fever. The *fastigium*, if present at all, was less than one day in length.

The period of defervescence was about three days long, extending from between the 31st and 32d to between the 34th and 35th. The temperature decreased with fair regularity to below normal on the last day.

The period of convalescence was characterized by a sharp drop in temperature to subnormal during the first day. The next three days it rose to about normal, from which point it gradually decreased until death occurred, which was between the fifth and sixth days of this period.

The diurnal variations: At five o'clock on the afternoons of the 29th, 30th, 31st, 32d, and 35th days after inoculation, the temperature was 106.3°, 106.0°, 106.0°, 106.2° and 100.9° respectively. The morning temperatures for the same days were 103.2°, 105.0°, 105.2°, 104.0°, and 98.3°. This shows an irregular diurnal variation of 1.0° to 3.1°. In healthy cows of the same age the diurnal variation is only about 1° to 2°.[1] Accordingly the term *continued remittent* might appropriately be used to describe the fever.

THE BLOOD.

The blood sediment normally amounted to 36.0 per cent. On the third day after inoculation no change was observed. A marked decrease was seen on the sixth, from which time until the 10th the decrease was very slight and gradual.

The observation made on the 12th showed a sharp decline. From this date until the 15th, the decrease was very slight. On the 16th, another sharp drop occurred and the minimum for the primary fever period was reached (20.0 per cent). From this point until the 20th on the whole a slight increase occurred. The 21st showed a marked increase which continued more gradually until the 23d, the day on which the maximum was observed for the secondary incubation period (24.5 per cent). From this date was observed a regular marked decrease until the 34th, at which time the minimum for the secondary fever period (nine per cent) was reached. Until the 36th, a slight increase occurred, succeeded by a fall which continued until the 38th, when the amount of sediment was the same as on the 34th. No further change was observed.

The number of red corpuscles decreased until the 10th day coincidently with, and was proportional to the decrease in the blood sediment as evidenced by the volume indices.

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[1] CONNAWAY, *Mo. Exp. Sta. Bull.*, 1897, 37, p. 106.

The decrease shown on the 10th day in the number is greater than the hematokrit reading would indicate. Of course when the average volume of the corpuscles is altered as in the disease under discussion the hematokrit reading ceases to be a reliable criterion of the number.

For instance, if we take the normal number for this individual as 5,500,000 and the normal hematokrit reading as 36.0 per cent then, one per cent of sediment represents 150,000 corpuscles. On the 10th day, the hematokrit reading (31.0 per cent) indicated $31.0 \times 150{,}000 = 4{,}650{,}000$ corpuscles, while the actual count was 4,120,000.

The white count on this date showed a decrease also; therefore the increase in the total volume could only have been brought about by an increase in the individual size of the red corpuscles, or by the presence of suspended matter in the blood other than intact formed elements. Careful microscopical examination yields no evidence to support the latter hypothesis. A general increase in the size of the corpuscles is therefore the most probable explanation, as the microscope did not show any megalocytosis at this time.

Three days later, the 13th day of the disease, and next to the last day of the incubation period, the number of red cells remained unchanged, but the hematokrit showed a decrease to 25.5 per cent. A large increase in the number of the white cells occurred during this period, but this alone will not account for all the loss in sediment. The volume index shows that the average size of the red cells is slightly less than normal.

On the 16th day of inoculation the count was still greater, relative to the hematokrit reading, although the white cells had decreased almost 50 per cent since the last observation. The red cells must therefore have continued to decrease in volume.

On the 17th day, the number of the reds had increased greatly and the hematokrit reading slightly, the whites remaining practically unchanged. The volume index sharply declined to 0.43, the minimum observed.

The number of corpuscles and volume index on the 23d were again the same as at the beginning of the primary fever period. This corresponds to the middle portion of the secondary incubation period. The next count made on the 36th day, corresponding to the beginning of the preconvalescent stage, showed the lowest number (1,026,000) and largest volume index (1.075) observed.

The relatively large volume index is explained in part, at any rate, by the morphological changes seen at this time (megalocytosis and relative increase in the number of the larger varieties of white corpuscles).

On the 37th, the day the last count was made, there was a slight increase in the number of red corpuscles and a decrease in the volume index. This corresponds to the last elevation of temperature. The morphological findings were practically the same as the day before.

On the whole then it would seem that from after the onset of the fever to its termination, the volume index of the red corpuscles is sub-normal. Table 4 and the following protocols, giving data obtained during 1901, substantiate this conclusion.

TABLE 4.

Day of Inoculation	Temperature 7 A. M.	Per Cent of Blood Sediment	Number Red Corpuscles per cu. mm.	Volume Index of Red Corpuscles
6	102.5	32.0		
to				
15	106.6	10.0		
15	106.6	10.0		
to				
18	102.5	11.0		
19	102.7	11.0	3,015,000	0.37
20	105.5	15.0		
21	105.5	16.0	3,105,000	0.50
22	104.6	14.0	2,461,000	0.51
23	103.1	15.0		
24	102.9			
25	103.1	15.0	2,402,000	0.62
26	102.8	16.0	3,508,000	0.43
27	105.8	17.0	3,762,000	0.40
28	103.7	17.5		
29	102.3	18.0		
to				
39	100.6	25.0		

Bull 4, Group 1.

Heifer No. 21 (Group 3) showed a primary fever period from the 17th to 25th day, the temperature rising to 105.0 on the 19th. On the 20th it was the same, thus giving a fastigium two days in length. The first day of defervescence, the temperature was 103.5; the count 6,960,000 and the blood sediment 30.0 per cent, giving a volume index of 0.43.

Heifer No. 15 (Group 3) had a very short primary fever lasting from the 19th to 23d day, The secondary fever began on the 32d day. The maximum temperature during the primary fever (104.3) occurred on the 21st day. The blood sediment increased from 25.0 on the 20th to 40.0 per cent on the 24th. The number of red corpuscles on the latter date was 7,575,000 giving a volume index of 0.52.

The increase in the volume index which occurred on the 10th can only be due to an increase in the average size of the corpuscles. This swelling might be occasioned by a decrease in the concentration of the blood plasma. Unfortunately, no freezing point determinations were made on this date. Changes in the permeability of the envelope of the corpuscles might account for the increase in volume, even though the osmotic pressure of the plasma remained unchanged. It is conceivable that such a change in the envelopes could result mechanically from the presence of the causative organism or chemically from products of its metabolism; or from specific lytic substances produced by the tissues of the host as the result of the presence of the organism or its products; or to the action of other substances, such as bile salts, present in the blood in sufficient quantity to exert a hemolytic action, the first stage of which, in general, is swelling.

The sharp increase in the number and the decrease in volume at the beginning of the fastigium is most probably due to a change in the total volume of the plasma, or a reversal of the changes in the envelope indicated above, and not to any change in the molecular concentration of the plasma, as the freezing point was practically unaltered. With the exception of a decrease in the number of free nuclei, shown by a count with a high power (14,400 per cu. mm. as against 20,000 on the preceding day), no morphological changes occurred. As there were many microcytes present and a high power was not used in counting the corpuscles it is not improbable that some of the free nuclei may have been mistaken for corpuscles. For this reason the red counts may be somewhat inaccurate. The apparent decrease in the volume index is probably partly explained in this way. The animal died three days later without any more counts being made.

The decrease in the number of white corpuscles began slightly prior to the beginning of the decrease in the number of the red corpuscles and in the blood sediment, that is between the first and third days. The decrease on the third day amounted to about 28 per cent of the total number. This decrease continues until the 17th which corresponds to the onset of the fastigium of the primary fever period. A decrease occurred between the 16th and 17th days. It will be remembered that on this day a sharp increase occurred in the number of the red corpuscles. The next count made on the 23d, corresponding to the intermediate period, showed an increase in the number existing between the first and third days. The next and last count, made on the 37th day showed a further decrease.

The proportion of the white to the red corpuscles decreased until the 17th day (3.1:1173 to 1:3407). The next count on the 23d showed a return to almost normal proportions (1:1099). The proportion on the 37th day was hypernormal (1:606). Thus it appears that the actual number of white corpuscles is decreased throughout the incubation and primary fever period; that it approaches normal during the intermediate stage and that during the secondary period it probably again decreases as the number taken on the 37th day shows a marked decrease from that of the intermediate period.

The percentage of hemoglobin begins to decrease between the third and sixth day. The decrease is gradual, but marked until the 16th day. A slight increase occurs on the 17th day corresponding to the onset of the fastigium of the primary fever period. A sharp increase was found on the 23d, which corresponds to the intermediate period. Just after the secondary fever period (37th day) a lower percentage of hemoglobin (about 20.0 per cent of normal) was found than was observed at any other stage. In all probability the percentage was still lower just prior to the death of the animal.

Most clinical observers agree that the Tallquist method gives a reading somewhat higher than the amount of hemoglobin actually present. The reading by this method averaged about 28° higher on the scale than by the method of Dare. The estimation based on the specific gravity of the blood showed a maximum increase in the hemoglobin amounting to 50 per cent.

In the blood sediment Hb ratio, we get a general increase to the 16th day and then a slight decrease to the 23d day, but still it was somewhat above the normal. On the 36th day, the amount of hemoglobin per unit of sediment was considerably below normal. This agrees with the microscopical appearances and the general conclusions drawn from the variatious in the color index in similar conditions of the blood in man.

The specific gravity of the blood was markedly decreased on the 17th and still more on the 37th day. The specific gravity of the serum was slightly less than normal on the 17th. On the 40th day the serum had normal specific gravity. Judging from the quantitative analysis on the 37th and 40th, the specific gravity on the 37th could not have been far from normal. The specific gravity of the serum therefore varies but slightly during the disease.

The osmotic resistance of the red corpuscles slightly increased, the beginning of which was between the 8th and 17th day. A further slight increase was seen in blood taken from the heart at postmortem.

The coagulation time of the blood which on the day of inoculation was 168 seconds was markedly increased on the 17th to 315 seconds, and on the 37th it was still further increased to 350 seconds.

The amount of fibrin was much less than normal on the 17th, but on the 37th it was more than normal.

Obviously, then, the coagulation time is not proportional to the amount of fibrin. The most plausible explanation of the increase in coagulation time is that the fibrin ferment was slow in forming owing to the decrease in the actual number of, and abnormal proportion of kinds, of white corpuscles. Also the greater concentration of the plasma and the presence of bile salts may have played a part.

The amount of coagulable proteids was determined quantitatively in serum from blood drawn on the day of inoculation and in serum from blood taken at the postmortem. A marked decrease was seen on the 40th day, a decrease so great that it could not in any case be explained as due to inaccuracies of the method used in estimating.

The alkalinity of the defibrinated blood was slightly higher than of the serum, on the day of inoculation, the 17th and the 37th days.

The alkalinity of each was less on the 37th than on the 17th.

The chlorides of the serum were increased during the disease, being about 20 per cent higher on the 37th day and about 40 per cent higher on the 40th, than on the day of inoculation.

It is unfortunate that the determination was overlooked on the 17th day.

The diastatic ferment of the blood is decreased during the disease. The amount present on the 17th was less than on the 37th day. The serum contained more ferment per c.cm. than the defibrinated blood.

Freezing point observations on the blood and serum were the same on the 17th as on the day of inoculation. On the 37th, the Δ of the serum was increased by 0.107° and that of the blood by 0.097°. From this it is evident that a marked concentration of the plasma occurred between the 17th and 37th days. All observations showed a slightly greater Δ for the serum than for the defibrinated blood. On the 17th, the difference was only 0.003°, which is quite within the error of observation. But on the 37th the difference was 0.013° and on the day of inoculation 0.009°, while the differences found in successive observations on one and the same serum or blood never exceeded, as has been previously stated, 0.005° and were usually not more than 0.003°

The electrical conductivity of the blood showed an increase on the 17th day, due doubtless to decrease in the total volume of corpuscles. The conductivity of the serum was unchanged. On the 37th day there was a further increase of the conductivity of the blood. This, of course, was also largely due to diminution in the total volume of the corpuscles. But it must also be attributed in part to the marked increase in the conductivity of the serum, which is associated with the increase in chlorides (and possibly other inorganic salts). The decrease in coagulable proteids also must of course cause an increase in the conductivity of the serum, since non-electrolytes depress the conductivity in proportion to their concentration.

Bile salts were demonstrable in the serum from blood taken on the 37th day by the following method:

Twenty-five c.cm. of serum were added to 75 c.cm. of 95 per cent alcohol and carefully sealed to prevent evaporation. Some months later, the mixture was filtered and the filtrate tested by evaporating two to three c.cm. in a porcelain dish and testing the residue by Pettenkofer's method. The result was positive, a very faint purple stain being obtained. The alcoholic filtrate gave no visible rotation to the plane of polarized light when tested in a polariscope.

THE URINE.

The specific gravity gradually decreased from 1.032 on the third to 1.009 on the 37th day owing to a decrease mainly in the percentage of inorganic solids. On the 40th day it was 1.027.

The alkalinity increased to five times its original amount between the third and 17th days. A marked decrease was observed on the 19th day and on the 38th an increase again but not to the same degree as on the 17th day. On the 40th it was again markedly decreased.

The chlorides decreased irregularly from the third to the 19th day. The first decrease was observed on the 13th. No further change occurred to the 17th day. Two days later (the 19th) an additional decrease was found. On the 38th the amount was practically the same as on the third day. An increase to above the original amount was found on the 40th day.

The Urea showed an increase the third and 13th days. On the 19th and 38th days it was decreased slightly, the amounts on the two days being practically the same. The 40th day showed a further decrease.

Coagulable proteids were not demonstrable on the 3d, 13th, 17th or 19th days. Amounts equivalent to 0.165 and 0.330 per cents were found on the 38th and 40th days respectively.

The Δ (*depression of freezing point*) was 0.485° less on the 17th and 1.081° less the 38th than on the third day. Since electrical conductivity was diminished this was probably largely due in both instances to a decrease in electrolytes.

The relative electrical conductivity showed a very marked decrease on the 17th and a still greater decrease on the 38th as compared with the third day.

The diminution in the chlorides explains a portion of this decrease but is not sufficient to account for it all. The presence of albumin on the 38th would act in the same direction but since a gram of albumin in 100 c.cm. of urine would only diminish the conductivity by about two per cent, a considerable portion of diminution of conductivity must be due to a decrease in electrolytes other than chlorides.

Up to the 40th day, no blood pigment was observed in the urine. On this day, when examined in a thick layer by transmitted light, a reddish color was seen. Spectroscopic examination showed a single faint band corresponding in position to that of reduced hemoglobin.

The morphology of the blood in Cow 2 was studied in fresh blood before drying and in film preparations made by spreading the blood thinly on cover-glasses, drying in the air, fixing by heat, staining with alcoholic eosin and alkaline methylene blue, drying and mounting in Canada balsam.

The following notes were made:

On day of inoculation.—Red cells of good color and well formed. A few are larger than the average, but the latter predominate. The white cells are mainly basophile, consisting largely of lymphocytes and eosinophile leucocytes.

Third day.—Red cells unchanged. White cells appear to show slight increase in eosinophile leucocytes.

Sixth day.—Red cells unchanged. White cells show relative predominance of lymphocytes.

Tenth day.—Red cells show no obvious change. White cells in many cases do not appear normal. Of five eosinophile leucocytes observed, four were in various stages of disintegration. They appear to be more numerous than on the sixth. Also a few groups of free nuclei, evidently once belonging to polynuclear white cells were seen.

Thirteenth day.—Red cells unchanged. White cells apparently show a small relative increase in the polynuclear basophile leucocytes.

Sixteenth day.—Red cells not obviously changed. The white cells show marked changes. Many of the polynuclear leucocytes of the slightly basophile variety showing a kind of unipolar staining with eosin, were seen. Also cells of the larger polynuclear variety with the protoplasm distinctly and evenly stained with eosin were observed. These abnormal types together with the eosinophile leucocytes represent from 40 to 60 per cent of the total number of the white cells, the remainder being mainly lymphocytes.

Seventeenth day.—The red cells apparently show fewer megalocytes than on the 16th; a considerable number of microcytes were present. White cells very few in number. Lymphocytes appear to predominate.

Twenty-first day.—Red cells appear same as before. Possibly fewer microcytes. White cells, no change observed.

Twenty-third day.—Red cells same as on 21st. No nucleated reds observed up to this time. White cells appear same as on 21st.

Thirty-sixth day.—Red cells show marked poikilocytosis. About 20 per cent are nucleated and many free nuclei are present. The cells stain poorly with eosin. White cells more numerous in proportion to the reds. Apparently the lymphocytes predominate. Many eosinophile and basophile polynuclear varieties are present.

Thirty-seventh day.—Red cells same as last time or poikilocytosis is more marked. White cells appear same as before.

The postmortem was made within two hours of the death of the animal. The findings were characteristic of the disease.[1]

Specimens from all the visceral organs were preserved and subsequently embedded, sectioned, stained and mounted, but space forbids a presentation of the results here.

A fetus about four months old was removed from the uterus. Blood obtained by cutting the umbilical vessels was subcutaneously injected into an animal susceptible to the fever. The result was negative. Amniotic fluid was secured and on examination gave the following results :

Color, clear light yellow.

Sp. Gr., 1.009.

Alkalinity, 28.0 mg ms. NaOH per 100 c.cm.

Urea, 0.0027 gms. per c.cm.

Chlorides, 0.68 gms. per 100 c.cm.

Coagulable proteids, negative.

Reducing power for Fehlings solution equivalent to 0.0006 gms. dextrose per c.cm.

Spectroscopic examination, negative.

With a view of determining if isolysins or autolysins were produced by the disease, the following experiments were made:

On the day of inoculation, the sera from the three cows were carefully examined for isolysins. In order to be sure of getting a reaction if any active substance was present, tubes containing equal parts of serum and five per cent suspension of washed corpuscles in nine-tenths NaCl were employed. To save space the results are not given in detail. The following conclusions were arrived at:

1. Serum of Cow 1 was slightly hemolytic for the washed corpuscles of Cows 2 and 3.
2. Serum of 2 was slightly hemolytic for the washed corpuscles of 3, but not for 1.
3. Serum of 3 was markedly hemolytic for the washed corpuscles of 2, but not for 1.

Control tubes of serum and corpuscles from one and the same animal gave negative results in each case.

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[1] CONNAWAY, *Mo. Exp. Sta. Bul.*, 1897, 37, p. 90.

The time of "spontaneous" or "auto" laking was determined by putting five c.cm. of sterile defibrinated blood from each animal on the day of inoculation into sterile test tubes which were plugged with sterile cotton and kept in an amber glass bottle at 25° C. No laking occurred until the third day, when the blood from Cows 2 and 3 showed slight laking, the latter a little more than the former. At this time that from Cow 1 was unchanged. Of course the corpuscles settled to the bottom of the tube and laking was readily detected by observing the clear serum at the zone of contact with the sediment.

On the fourth day, the blood of Cow 1 was still unlaked.

On the eighth day, the tubes from cows 1 and 2 showed moderate laking while that from Cow 3 was strongly laked. Microscopical examination showed no bacteria and there was no putrifactive odor.

On the 17th day after inoculation blood from Cow 2 was tested again in the same manner. No laking was visible four days later, the 21st day. Again on the 37th day, the test was made on Cow 2. Three days later, the 40th, a very slight amount of laking was visible on close inspection.

Blood obtained from the heart at postmortem by aseptic technique and tested in the same way, was not laked 24 hours later.

From this it may be safely concluded that the time of "spontaneous" laking was not greatly altered in Cow 2.

It is hoped that an opportunity may be had to extend these observations on animals having the severer symptoms of the disease.

It has been stated that blood from an immune cow kept aseptically for several days will produce the disease on inoculation into a susceptible animal, and that such aseptic blood shows extra-corpuscular forms of the specific plasmodium. I therefore inoculated defibrinated blood from Cow 1 (the immune) kept aseptically as 25° C. for 14 days into a susceptible animal but obtained a negative result notwithstanding the fact that, microscopically, this blood after incubation showed numerous bodies corresponding to the descriptions and plates of the extra-corpuscular forms above mentioned.

Further investigations along this line are yet necessary and will be prosecuted at the first opportunity.

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